Clinical Trial Protocol

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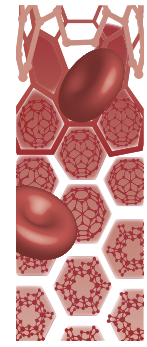

A phase Ib/II trial of PEP503 (NBTXR3, radioenhancer) with radiotherapy and chemotherapy in patients with rectal cancer

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Aims: To investigate the safety profile, dose-limiting toxicity and antitumor activity of PEP503 (NBTXR3) nanoparticles with radiotherapy and concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer. **Methods:** Patients will receive a single intratumoral injection of the nanoparticles, followed by radiotherapy and intravenous infusion of fluorouracil or oral capecitabine concurrently. In phase Ib (escalation phase, 3 + 3 design), volume escalation is based on the tumor volume of 5%, 10%, 15% and 22% of total baseline tumor volume. In phase II (expansion phase), 18 additional patients will be enrolled. **Discussion:** This study will be the first prospective, open-label, single-arm, nonrandomized study to investigate the efficacy and safety profile of PEP503 (NBTXR3) nanoparticles with radiotherapy and chemotherapy in these patients.

Trial registration number: NCT02465593 (ClinicalTrials.gov)

Plain language summary: Preoperative concurrent chemoradiotherapy is the standard treatment for patients with locally advanced rectal cancer. PEP503 (NBTXR3) has radioenhancement properties. Therefore, the dose per fraction during radiotherapy could be reduced, and the same therapeutic efficacy could be retained when PEP503 (NBTXR3) nanoparticles are used during radiotherapy. This study reveals the protocol of a phase Ib/II study to investigate the safety profile, dose-limiting toxicity and antitumor activity of PEP503 (NBTXR3) nanoparticles with radiotherapy combined with concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer.

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Keywords: intratumoral injection • locally advanced rectal cancer • PEP503 (NBTXR3) nanoparticle • radioenhancer • radiotherapy • unresectable rectal cancer

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Introduction to the trial

The title of this article was 'A phase Ib/II trial of PEP503 (NBTXR3, radioenhancer) with radiotherapy and chemotherapy in patients with rectal cancer'. The study is funded by PharmaEngine, Inc. (Taipei, Taiwan). The study has two parts: escalation phase (part Ib) and expansion phase (part II). The patients with confirmed rectum adenocarcinoma (T3-4, N any or locally unresectable tumor with a distal tumor margin ≤10 cm from the anal verge) without evidence of distant metastatic disease will be enrolled. The product under investigation is PEP503 (NBTXR3) nanoparticles, which are a radioenhancer. PEP503 (NBTXR3) nanoparticles have been reported to be taken up by various cancer cell lines and PEP503 (NBTXR3) nanoparticle injection with radiotherapy increased cancer cell death compared with radiotherapy alone. Therefore, the authors chose PEP503 (NBTXR3) nanoparticles for this study.

Background & rationale

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related death worldwide [1]. In 2020, approximately 1.88 million new CRC diagnoses and 915,000 CRC-related deaths were reported worldwide [1]. Since 2006 in Taiwan, CRC has been the most common cancer type, and its prevalence has increased rapidly. In 2009 and 2019, the incidence of CRC was 54.0 and 73.3 per 100,000 population, respectively (with 12,488 and 17,302 new diagnoses, respectively) [2]. Moreover, CRC is the third leading cause of cancer-related death in Taiwan. In 2021, 6657 people in Taiwan died because of CRC, with the mortality rate being 21.2 per 100,000 population in 2011 and 28.4 per 100,000 population in 2021 [2].

Despite substantial advancements in the treatment of localized malignancies, metastatic disease remains the primary cause of cancer morbidity and mortality. At least in some tumor types, the primary tumor seems to acquire metastatic traits during local progression at the primary site. The short latency of metastatic relapse in aggressive diseases implies that potent multiorgan metastatic competence either exists in premalignant cells or is present during the early stage of tumor invasion. Thus, local cancer control is crucial for anticancer treatment. For most patients with localized diseases, the primary anticancer therapy is surgical resection combined with radiotherapy. Radiotherapy is essential for rapid and effective cancer palliation. Furthermore, preoperative concurrent chemoradiotherapy (CCRT) confers survival benefits on patients with locally advanced cancer, including locally advanced rectal cancer [3–5]. It is, therefore, the standard treatment for patients with locally advanced rectal cancer. Several new radiotherapy modalities are been evaluated based on treatment planning and dose delivery defined strategies, including intensity-modulated radiotherapy (IMRT) and intensity-modulated arc therapy. The role of radiotherapy will continue to change in the coming years.

PEP503 (NBTXR3) [6–8] is a suspension of inert, crystalline nanoparticles of hafnium oxide, with hydrodynamic diameter distribution of 30–65 nm (volume distribution). Regardless of which linear accelerator is used as a source of ionizing radiation, these nanoparticles require activation by an external x-ray beam to generate oxygen free radicals to selectively destroy cancer cells. Hafnium oxide nanoparticles interact with a photon or an electron in an identical manner; therefore, they generate the same type of effect as water molecules, but the effect is greater by several orders of magnitude [6]. PEP503 (NBTXR3) nanoparticles are therefore a potential radioenhancer [6,7]. Any equipment implemented in clinical settings can be used to activate PEP503 (NBTXR3) nanoparticles.

PEP503 (NBTXR3) nanoparticles are intended for cancer therapeutics. They are a biocompatible product designed to be activated by ionizing radiation to kill cancer cells and achieve tumor control. The appropriate use of PEP503 (NBTXR3) nanoparticles designed for therapeutic application requires an assessment of cell type-specific sensitivity in both noncancer and cancer cells. Furthermore, explorations of the dose–response relationship requires that PEP503 (NBTXR3) nanoparticles be tested at increasing concentrations to determine the optimal level of nanoparticles for their use in *in vitro* models.

PEP503 (NBTXR3) nanoparticles have been reported to be taken up by various cancer cell lines, including CRC, glioblastoma, lung cancer, breast cancer, prostate cancer and fibrosarcoma [7–9] and the uptake level depends on the cell line. The cellular uptake of such nanoparticles is mediated by endocytosis in a concentration-dependent manner, and they then form clusters in the cytoplasm [7–10]. PEP503 (NBTXR3) nanoparticle injection with radiotherapy has increased cancer cell death compared with radiotherapy alone [7–11]. Therefore, PEP503 (NBTXR3) nanoparticles can help reduce the dose per fraction during radiotherapy but retain the same therapeutic efficacy. Zhang *et al.* demonstrated that PEP503 (NBTXR3) nanoparticles with radiotherapy could produce a significant abscopal effect

in vitro to increase local and distant tumor control, significantly lengthening the lifespan of mice [9]. Zhang *et al.* demonstrated that the aforementioned combination can control tumor growth *in vivo*, including tongue cancer, prostate cancer, pharynx cancer, fibrosarcoma, liposarcoma and lung cancer [10].

PEP503 (NBTXR3) nanoparticles have been designed for a single-dose intratumoral injection. In studies, after injection, such nanoparticles were well dispersed and retained within tumors for the duration of radiotherapy *in vivo* [6,7], moreover without marked leakage to surrounding normal tissue [6,7].

The potency of high-density material such as hafnium oxide has been well determined in *in vitro* and *in vivo* models. After injection, PEP503 (NBTXR3) nanoparticles disperse well within the tumor mass and reside there for more than 2 weeks [6,7]. This property is highly relevant because, for most cancers, radiotherapy is delivered in daily doses over a period of 4–7 weeks. NBTXR3 nanoparticles were used in a phase I clinical trial conducted by Nanobiotix involving 12 patients with locally advanced soft tissue sarcomas (ClinicalTrials.gov, registration number NCT01433068). In another phase I clinical trial, clinicians administered intratumoral injections of NBTXR3 nanoparticles in 19 patients with locally advanced squamous cell carcinoma of the oral cavity or oropharynx (ClinicalTrials.gov, registration number NCT01946867) [12]. In a phase II–III clinical trial, clinicians administered such nanoparticles to patients with locally advanced soft tissue sarcomas (ClinicalTrials.gov registration number, NCT02379845) [13]. However, no clinical study has investigated their therapeutic efficacy in patients with rectal cancer. In this phase Ib/II study, the authors plan to investigate the safety profile, dose-limiting toxicity (DLT) and antitumor activity of PEP503 (NBTXR3) nanoparticles with radiotherapy in combination with concurrent chemotherapy for patients with locally advanced or unresectable rectal cancer.

Design

Overall study design

The present study is a prospective, open-label, single-arm, nonrandomized study for evaluating the effects of PEP503 (NBTXR3) nanoparticles in locally advanced or unresectable rectal cancer. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-2013-11-07[II]), Taichung Veterans General Hospital (SF18357A) and Taipei Medical University Hospital (N202006048). The study is registered with ClinicalTrials.gov (NCT02465593).

The study has two parts:

1. Escalation phase (part Ib): a 3 + 3 dose-escalation study design will be adopted in this phase to identify the recommended volume of PEP503 (NBTXR3) nanoparticles for intratumoral injection.
2. Expansion phase (part II): following confirmation of the recommended volume of intratumoral injection, 18 additional patients will be enrolled at the recommended volume level for evaluations of antitumor efficacy.

The target population is patients with confirmed rectum adenocarcinoma (T3–4, N any or locally unresectable tumor with a distal tumor margin ≤10 cm from the anal verge) without evidence of distant metastatic disease, with an Eastern Cooperative Oncology Group performance score 0–1 and with adequate bone marrow, renal and hepatic function.

Enrollment schedule

In the phase Ib portion (dose-escalation phase), patient enrollment will be based on the traditional 3 + 3 design in which the number of patients enrolled is based on the DLT that occurred in each cohort.

In the phase II portion (expansion phase), patient enrollment will start after the recommended volume of phase Ib is confirmed. 18 patients will be enrolled continuously in this phase and will receive PEP503 (NBTXR3) nanoparticles at the recommended volume identified in phase Ib.

Study end points

Primary end point

Phase Ib

1. To assess the safety profile and determination of the DLT of PEP503 (NBTXR3) nanoparticles administered intratumorally and activated by external-beam radiation with concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer.

2. To define the recommended volume (dose) of PEP503 (NBTXR3) nanoparticles administered intratumorally and activated by external-beam radiation with concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer.

Phase II

To evaluate the antitumor activity in terms of the response rate, as per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 [14] of PEP503 (NBTXR3) nanoparticles at the recommended volume administered intratumorally and activated by external-beam radiation with concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer.

Secondary end point

Phase Ib

To characterize the pharmacokinetic profile of PEP503 (NBTXR3) nanoparticles after intratumoral injection.

Phase II

1. To evaluate the antitumor activity of PEP503 (NBTXR3) nanoparticles administered intratumorally and activated by external-beam radiation in combination with chemotherapy in patients with locally advanced or unresectable rectal cancer in terms of the objective response rate, complete resection rate (R0 resection rate), pathological complete response rate, pathological response rate, local or locoregional recurrence rate at 1 year, distant recurrence rate at 1 year and disease-free survival rate at 1 year.
2. To evaluate the safety profile of PEP503 (NBTXR3) nanoparticles administered intratumorally and activated by external bean-radiation combined with chemotherapy in patients with locally advanced or unresectable rectal cancer.

Study population

Inclusion criteria

1. Histologically proven rectum adenocarcinoma (T3-4, N any or locally unresectable disease, without evidence of distant metastases [M0]).
2. Distant tumor margin located ≤10 cm from the anal verge.
3. Stage and resectability assessment with magnetic resonance imaging (MRI), transrectal ultrasound or computed tomography (CT) scan.
4. Eastern Cooperative Oncology Group performance score 0–1.
5. Age: 20–80 years old.
6. Adequate bone marrow, renal and hepatic function as follows:

- Absolute neutrophil count of ≥1500/mm^3;
- Platelet count of ≥100,000/mm^3;
- Total bilirubin of ≤1.5× the upper limit of normal (ULN);
- Aspartate aminotransferase and alanine aminotransferase levels of ≤2.5× ULN;
- Alkaline phosphatase level of ≤2.5× ULN;
- Calculated creatinine clearance of ≥50 ml/min or creatinine level within the normal range.

1. All women of childbearing potential must have a negative urine pregnancy test within 7 days prior to study treatment with PEP503 (NBTXR3) nanoparticles. Patients must agree to use effective contraception during the study.

Exclusion criteria

1. Prior history of pelvic radiotherapy.
2. Hypersensitivity to fluoropyrimidine.
3. Uncontrolled serious medical or psychiatric illness.
4. Myocardial infarction or uncontrolled angina pectoris within the previous 6 months.
5. No more than 4 weeks since prior participation in any investigational drug study.
6. Major surgery within 28 days.

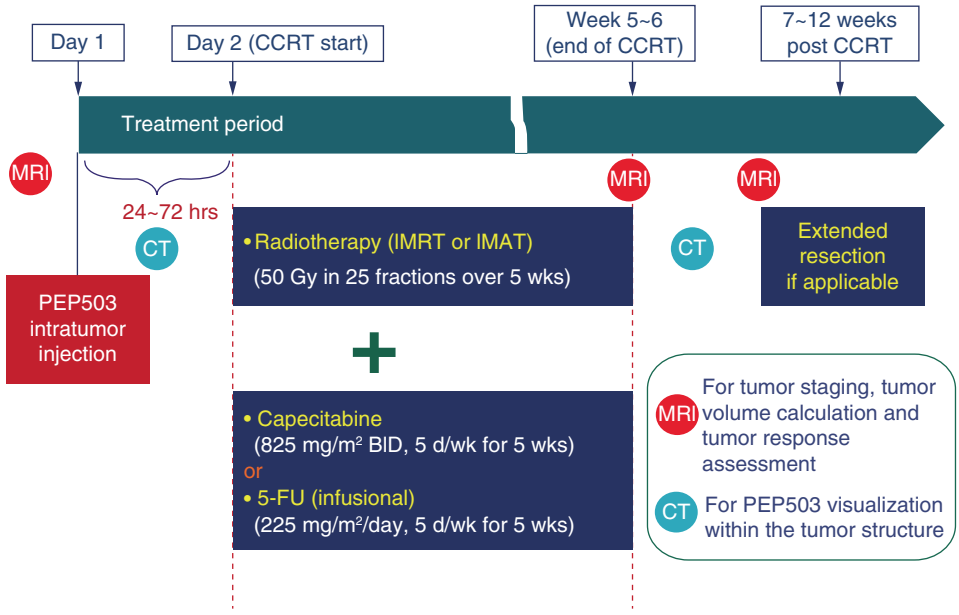

Figure 1. Study flowchart.
BID: Twice a day; CCRT: Concurrent chemoradiotherapy; IMAT: Intensity-modulated radiotherapy; IMRT: Intensity-modulated arc therapy; MRI: Magnetic resonance imaging.

7. Other malignancies within the past 3 years except for effectively treated squamous cell or basal cell skin cancer, melanoma *in situ*, carcinoma *in situ* of the cervix or carcinoma *in situ* of the colon or rectum.
8. Cardiovascular disease that would preclude study treatment or follow-up.
9. Informed consent for study participation not signed.

Number of participants

1. Phase Ib: Approximately 3–24 patients are expected to be enrolled. The sample size may vary depending on feasibility, observed DLTs and the resultant injection volume.
2. Phase II: Following confirmation of the recommended volume, 18 additional patients will be enrolled to receive injections at this volume level; thus, 21–42 patients will be treated in this study. The sample size is arbitrarily decided due to the lack of available data to estimate the sample size for this exploratory study.

Treatment plan

After oral or intravenous corticosteroid, PEP503 (NBTXR3) nanoparticles will be administered intratumorally on day 1, followed by radiotherapy 24–72 h later – IMRT or intensity-modulated arc therapy (IMAT) of 5000 centigray (cGy) in 25 fractions (200 cGy/fraction, five-times/week) – to the gross tumor and involved nodes. Patients will receive concurrent intravenous infusion of fluorouracil (5-FU; 225 mg/m^2 a day, 5 days/week for 5 weeks during the radiotherapy period) or oral capecitabine (825 mg/m^2 twice a day 5 days/week for 5 weeks during the radiotherapy period). Once the CCRT is completed, patients have recovered from the effects of radiation (~8 weeks after the completion of chemoradiotherapy) and the tumor has become resectable, tumor resection will be performed (Figure 1).

Volume escalation of PEP503 (NBTXR3) nanoparticles

The classical 3 + 3 design for volume escalation is the basis of this trial involving four volume levels of PEP503 (NBTXR3) nanoparticles. The volume levels are based on the tumor volume of each patient, with levels 1, 2, 3 and 4 corresponding to 5, 10, 15 and 22% of the total baseline tumor volume, respectively. PEP503 (NBTXR3) nanoparticles will be administered at a fixed concentration of 53.3 g/l. The baseline tumor mass volume will be measured according to MRI results, and dosimetry planning will involve CT scan (Table 1).

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Table 1. Volume escalation of PEP503 (NBTXR3) nanoparticles.

Volume level	Injection volume (% baseline tumor volume)	PEP503 concentration (g/l)	Design
Level 1	5	53.3	3 + 3
Level 2	10		
Level 3	15		
Level 4	22		

Dose-limiting toxicity

The DLTs are the following toxicities that occur in the period from the administration of PEP503 (NBTXR3) nanoparticles to 4 weeks after the radiation treatment completion.

1. Any grade ≥3 adverse events (AEs) scored by the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0 (NCI-CTCAE v. 4.0) [15]; this scoring is reasonably related to the product in combination with radiotherapy during the period from the administration of PEP503 (NBTXR3) nanoparticles to 4 weeks after the completion of radiotherapy.
2. Any other toxicity reasonably related to the product and any toxicity the investigator or the sponsor's medical expert deems to be dose limiting, regardless of the grade, may be considered as a DLT.
3. AEs with unusual duration that are reasonably related to the product, regardless of severity, may be considered as a DLT after the evaluation of clinical course and unusual outcomes.

Procedure of intratumoral injection of PEP503 (NBTXR3) nanoparticles

Before injection, the authors calculated the injection numbers (each injection amount ≦0.2 ml) and selected the injection sites according to the shape of the tumor to evenly distribute the PEP503 (NBTXR3) nanoparticles. The depth of the tumor on each injection site was measured by endoscopic ultrasonography. The length of the injection needle is 5 mm and the injection angle is usually tilted; therefore, the authors excluded the site on which the depth of the tumor was ≦5 mm to prevent perforation. After the injection procedure, some bloody fluid from the anus was observed and it subsided the next day.

Volume escalation

At level 1, the patients will be screened and treated at the initial volume level of PEP503. When the first patient at the first dose level has received the test product, has been assessed for PEP503 (NBTXR3) nanoparticle tumor bioavailability, has received 3000 cGy of radiotherapy to the gross tumor (~3 weeks) with concurrent chemotherapy and has been evaluated for safety, the next two patients will be recruited. If no DLT is observed after the DLT evaluation periods of the three patients, the next group of patients will be treated at the higher volume level (level 2). In the event of a DLT event, 3 more patients will be treated at this level. If ttwo out of three to six patients exhibit ≥1 DLT, patient enrollment will be stopped at this initial level (level 1), and the study design will be reviewed. Conversely, if < one patient experiences a DLT (1 of 6), the next group of patients will be treated at the higher volume level (level 2).

At level 2, three patients will be treated and receive PEP503 through intratumoral injection with 10% of the baseline tumor volume. If no DLT is observed after the DLT evaluation periods, the next group of patients will be treated at the higher volume level (level 3). In the event of a DLT, three more patients will be treated at this level. If two out of three to six patients have >1 DLT, patient enrollment at this level will be stopped. Conversely, if < one patient experiences a DLT (1 of 6), the next group of patients will be treated at the higher volume level (level 3).

At level 3, a cohort of three patients will be treated and receive PEP503 intratumoral injection with 15% of the baseline tumor volume. If no DLT is observed after the DLT evaluation periods of the three patients, the next patients will be treated at the immediately higher volume level (level 4). In the case of 1 DLT, three more patients will be treated at this level. If two out of three to six patients have >1 DLT, patient enrollment at this level will be stopped. Conversely, if < one patient experiences a DLT (1 of 6), the next group of patients will be treated at the higher volume level (level 4).

At the highest planned level (level 4), three patients will be treated and receive an intratumoral injection of PEP503 (NBTXR3) nanoparticles with 22% of the baseline tumor volume. If no DLT is observed after the DLT

evaluation periods of the three patients, enrollment will be expanded up to six patients at this level. In the event of a DLT among the first three patients, three more patients will be treated at this level. If two out of three to six patients present have >1 DLT, patient enrollment at this level will stopped. If the volume injected at this level (level 4) is not tolerated, the volume level will revert to the lower level. Conversely, if < one patient experiences a DLT (1 of 6), the level 4 volume will be considered as the recommended one. A minimum of six patients are required to confirm the recommended volume. A volume level requires that < one of six patients develop a DLT to be confirmed as the recommended volume level. In phase II of this study, an additional 18 patients will be enrolled and treated with the recommended volume level. Intrapatient quantity increases (a higher volume or an additional injection) are not permitted. The PEP503 concentration to be injected in any case must remain equivalent to 53.3 g/l.

Investigated product

Formulation of PEP503 (NBTXR3) nanoparticles: sterile, white suspension at 64 g/l; pH range 6–8. Packaged in a 10 ml amber glass vial with a rubber (bromobuthyl) stopper and sealed with aluminum-center tear-off blue seal. Each vial contains 5 ml of PEP503 suspension.

Storage conditions

PEP503 (NBTXR3) nanoparticles should be maintained between 15 and 25°C and must not be refrigerated or frozen. PEP503 (NBTXR3) nanoparticles must be retained in the original package, in a locked area with restricted access, and handled per the manufacturer's instructions.

Assessments

Efficacy evaluations

The tumor will be biopsied for pathological examination (cancer diagnosis). After treatment, the resected tumor and lymph nodes will be evaluated for pathological response, and tumor responses will be assessed according to RECIST 1.1 after the completion of chemoradiotherapy.

Safety evaluations

The radiotherapy or fluoropyrimidine dose will be adjusted according to toxicities during the study treatment when applicable. AEs will be evaluated according to NCI-CTCAE v. 4.0.

Kinetics study (phase Ib only)

Blood and urine sampling for kinetics evaluation of PEP503 (NBTXR3) nanoparticles will be performed in the phase Ib portion. Seven blood and two urine samples will be collected from each patient who is willing to participate in the kinetics study. The blood samples will be taken before the injection, immediately after the onset of the injection, at the end of injection and 5 min, 10 min, 60 min and 4 h after the end of injection. Urine samples will be collected at the first and second voids after PEP503 (NBTXR3) nanoparticle intratumoral injection.

Intratumoral dispersion evaluation

CT scan images were taken before and after radiation and before tumorectomy.

Data management

An authorized contract research organization (contracted by PharmaEngine, A2 Healthcare, Taipei, Taiwan) will conduct data management. Data handling and analysis will be conducted in compliance with the International Committee of Harmonization and applicable regulatory guidelines. The data management contract research organization will conduct the study in compliance with the clinical study protocol, Good Clinical Practice, local and international applicable regulatory requirements and contract research organization standard operating procedures. The ultimate responsibility resides with the sponsor, PharmaEngine (Taipei, Taiwan).

The investigator or designee will enter the data collected using an electronic data capture system. Data will be collected from all patients who sign an informed consent form and are screened for the study regardless of whether they are subsequently randomized. For screening failures, minimal demographic data and reasons for screening failure will be collected.

The monitor should verify the data in the electronic case report forms (eCRFs) with source documents and confirm that no inconsistencies exist between them. The investigator or site designee will ensure that all data in

10.2217/nnm-2022-0186

the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site. A final database with the quality and integrity necessary for proper final reporting will be submitted for statistical analysis.

Statistical analysis

In phase Ib, only descriptive statistics will be used to summarize data. The actual sample size may vary depending on the injection feasibility, DLTs (if any) and the resultant treatment volume. Approximately 3–24 patients will be included and treated. The primary population for data analysis will be the 'all treated' population, with a specific focus on patients assessed for DLT and injection feasibility issues.

In phase II, an additional 18 patients will be enrolled and treated using the recommended volume to confirm the treatment's safety and evaluate its efficacy. The efficacy analysis will be based on the per-protocol population and all treatment populations at the recommended volume. The numbers of patients with objective response as per RECIST 1.1, a local radical resection rate (R0 resection rate), a pathological complete response rate and a pathological response rate will be tabulated. The 1-year local or locoregional recurrence rate, distant recurrence rate and disease-free survival rate will be determined at 1 year after the study.

The safety analysis will be based on patient exposure to PEP503. Analysis of intratumoral dispersion variables of PEP503 (NBTXR3) nanoparticle visualization will be performed as follows: CT scan evaluations of the nanoparticles in the tumor mass will be presented by the initial planned volume level of the nanoparticles (in tables) before and after PEP503 (NBTXR3) nanoparticle activation by radiotherapy and 8 ± 2 weeks after completion of the radiotherapy (before tumorectomy, if a radical resection of the primary tumor is applicable).

Furthermore, inductively coupled plasma mass spectrometry results will be tabulated for whole blood and urine samples according to the time point/tumor volume, injection feasibility concerns and initially planned volume level (1–4) of PEP503. Additionally, feasibility analysis will be presented by initial planned volume level, by patient characteristics and by baseline tumor volume and T stage.

Further treatment after surgery

The decision to administer postoperative adjuvant chemotherapy and the choice of regimen thereof will be at the investigator's discretion based on surgical pathology results and the relevant patient's medical condition. Information on postoperative adjuvant chemotherapy will be collected if available. Tumors may remain unresectable in some patients after treatment. Approximately in 10–25% of patients with unresectable rectal cancer, their cancers remain unresectable after preoperative CCRT. For patients unsuitable for radical surgery after treatment, palliative treatment as suggested by cancer treatment guidelines may be considered. However, the treatment administered to the patient will be at the treating physician's discretion based on the patient's medical condition and the hospital's standard practices.

Follow-up

The investigators should take all appropriate measures to ensure patient safety (Table 2); notably, they should investigate the outcome of any AEs (e.g., clinical signs and laboratory values) until the return to normal and monitor the patient's condition (including long-term safety data related to the investigational product or radiotherapy). In the case of any serious AE, the patient must be followed up until clinical recovery is complete and laboratory parameters have returned to normal/baseline or further anticancer treatment is administered due to locoregional or distant disease progression. This may imply that follow-up will continue after the patient has left the clinical trial (cutoff date of the study) and that additional investigations may be requested by the pharmacovigilance department.

Discontinuation

Treatment discontinuation

The study can be discontinued (including no other study therapy) for the following reasons:

1. In the investigator's opinion, continuation of the study treatment would be detrimental to the patient's wellbeing;
2. At the specific request of the sponsor;
3. Pregnancy;
4. Progressive disease;
5. Unacceptable toxicity.

Table 2. Flowchart for pretreatment and post-treatment investigations.

Period		Screening/baseline		PEP503 injection	Treatment period (week 1–week 5)‡‡‡				Follow-up (8 ± 2 weeks post-CCRT)				Radical resection§§§§	End of study¶¶¶¶	After study follow-up
Parameters	Day	≤28 days	≤7 days	Day 1	Day 2§§§	The 1st day of every week	Days 1–5 of every week	End of CCRT###	FU1	FU2	FU3††††	Tumor evaluation (end of follow-up)‡‡‡‡	Week 7–week 12 post-CCRT	2 weeks ± 3 days postsurgery	Every 3 months####
Informed consent†		X													
Demographics			X												
Patient's eligibility			X												
Medical history			X												
Physical exam			X	X	X	X			X	X	X	X	X	X	
Vital signs			X	X†††	X	X			X	X	X	X	X	X	
Height			X												
Weight			X	X†††	X	X			X	X	X	X	X	X	
Eastern Cooperative Oncology Group performance status			X	X	X	X			X	X	X	X	X	X	
MRI‡		X						X				X			X
PEP503 imaging (CT scan)§					X			X				X			
Hematology			X	X†††	X¶¶¶	X¶¶¶			X	X	X	X	X	X	
Biochemistry			X	X†††	X¶¶¶	X¶¶¶			X	X	X	X	X	X	
Urine analysis			X	X†††	X¶¶¶	X¶¶¶			X	X	X	X	X	X	
Tumor biopsy when not available within 3 months before day 1		X##													

†Within 28 days before day 1.
‡MRI result will be used for tumor volume calculation and tumor response (according to Response Evaluation Criteria in Solid Tumors v1.1 criteria) assessment. The same method and technique should be used to characterize identified lesions at baseline, during the study and at the follow-up period.
§CT scan for PEP503 visualization within the tumor structure (intratumor bioavailability) should be performed before the first fraction of radiotherapy (can be prior to day 2), after the completion of all the radiation and 8 ± 2 weeks after the completion of all radiation fractions (before the surgical resection).
¶Females with reproductive potential must have a negative urinary pregnancy test result within 7 days prior to the PEP503 intratumoral administration, to be repeated as clinically indicated.
#An ECG will be performed at screening, before the first fraction of radiation, after 15 fractions of radiations (after 3 weeks of radiotherapy), after all the fractions of radiation and before surgery. It can be repeated during the study only if clinically indicated.
††Administer corticosteroids before the onset of PEP503 injection. Oral route: prednisone 30 mg, approximately 12 ± 2 h and 2 ± 1 h before the injection of PEP503 or intravenous: dexamethasone 20 mg before the injection of PEP503. Other corticosteroids with properties and dosing equivalence can also be used.
‡‡Concomitant medications will be recorded from 7 days prior to the PEP503 administration until the end-of-study visit or resolution of ongoing study product-related adverse events, whichever is later.
§§Seven blood samples will be obtained from all patients on day 1. Whole blood will be sampled before (-24 h), immediately after the onset of PEP503 intratumoral injection (+10 min), at the end of the injection (+5 min) and 5 min (+5 min), 10 min (+10 min), 60 min (+10 min) and 4 h (+30 min) after the end of injection.
¶¶Urine samples will be collected at the first and second voids post-PEP503 intratumoral injection.
##If prior biopsy to confirm cancer diagnosis within 3 months prior to day 1 is not available.
†††No need to be repeated if within 7 days after baseline.
‡‡‡The CCRT period can last 6 weeks in order to complete 25 fractions of radiotherapy.
§§§Day 2 was defined as the day CCRT is administrated.
¶¶¶Sample should be collected before radiation.
###Will be performed within 1 week after the completion of CCRT.
††††This is an optional visit at the investigator's discretion based on the patients' condition.
‡‡‡‡For patients who are not candidates for radical resection after tumor evaluation, the tumor evaluation visit will be considered the end-of-study visit.
§§§§Only for patients who are candidates for tumor radical surgery. The radical resection will be arranged in the period from the 7th to the 12th week after the completion of CCRT. The international normalized ratio value will be monitored for the surgical procedure. Pathological response evaluation will be performed after tumor resection.
¶¶¶¶Patients who receive radical resection will be followed 2 weeks after surgery. This visit will be performed 2 weeks ± 3 days after surgery and patients will be evaluated with physical exam for vital signs, Eastern Cooperative Oncology Group performance status and adverse events.
####After-study follow-up will take place every 3 months ± 2 weeks and end 1 year after the end-of-study visit.
5-FU: Fluorouracil; CCRT: Concurrent chemoradiotherapy; FU1: First follow-up; FU2: Second follow-up; FU3: Third follow-up.

10.2217/nnm-2022-0186

Table 2. Flowchart for pretreatment and post-treatment investigations (cont.).

Period		Screening/baseline		PEP503 injection	Treatment period (week 1–week 5)‡‡‡				Follow-up (8 ± 2 weeks post-CCRT)				Radical resection§§§§	End of study¶¶¶¶	After study follow-up
Parameters	**Day**	**≤28 days**	**≤7 days**	**Day 1**	**Day 2§§§**	**The 1st day of every week**	**Days 1–5 of every week**	**End of CCRT###**	**FU1**	**FU2**	**FU3††††**	**Tumor evaluation (end of follow-up)‡‡‡‡**	**Week 7–week 12 post-CCRT**	**2 weeks ± 3 days postsurgery**	**Every 3 months####**
Urine pregnancy test¶			X												
ECG#			X		X	X#		X				X			
Administer PEP503				X											
Corticosteroids††				X											
Concomitant medications‡‡			X	X			X		X	X	X	X	X	X	
Blood kinetic study sampling§§				X											
Urine kinetic study sampling¶¶				X											
Adverse events				X	X		X		X	X	X	X	X	X	
Radiation therapy					X		X								
Chemotherapy					X		X								
Tumor radical resection													X		
Survival follow-up															X

†Within 28 days before day 1.
‡MRI result will be used for tumor volume calculation and tumor response (according to Response Evaluation Criteria in Solid Tumors v1.1 criteria) assessment. The same method and technique should be used to characterize identified lesions at baseline, during the study and at the follow-up period.
§CT scan for PEP503 visualization within the tumor structure (intratumor bioavailability) should be performed before the first fraction of radiotherapy (can be prior to day 2), after the completion of all the radiation and 8 ± 2 weeks after the completion of all radiation fractions (before the surgical resection).
¶Females with reproductive potential must have a negative urinary pregnancy test result within 7 days prior to the PEP503 intratumoral administration, to be repeated as clinically indicated.
#An ECG will be performed at screening, before the first fraction of radiation, after 15 fractions of radiations (after 3 weeks of radiotherapy), after all the fractions of radiation and before surgery. It can be repeated during the study only if clinically indicated.
††Administer corticosteroids before the onset of PEP503 injection. Oral route: prednisone 30 mg, approximately 12 ± 2 h and 2 ± 1 h before the injection of PEP503 or intravenous: dexamethasone 20 mg before the injection of PEP503. Other corticosteroids with properties and dosing equivalence can also be used.
‡‡Concomitant medications will be recorded from 7 days prior to the PEP503 administration until the end-of-study visit or resolution of ongoing study product-related adverse events, whichever is later.
§§Seven blood samples will be obtained from all patients on day 1. Whole blood will be sampled before (-24 h), immediately after the onset of PEP503 intratumoral injection (+10 min), at the end of the injection (+5 min) and 5 min (+5 min), 10 min (+10 min), 60 min (+10 min) and 4 h (+30 min) after the end of injection.
¶¶Urine samples will be collected at the first and second voids post-PEP503 intratumoral injection.
##If prior biopsy to confirm cancer diagnosis within 3 months prior to day 1 is not available.
†††No need to be repeated if within 7 days after baseline.
‡‡‡The CCRT period can last 6 weeks in order to complete 25 fractions of radiotherapy.
§§§Day 2 was defined as the day CCRT is administrated.
¶¶¶Sample should be collected before radiation.
###Will be performed within 1 week after the completion of CCRT.
††††This is an optional visit at the investigator's discretion based on the patients' condition.
‡‡‡‡For patients who are not candidates for radical resection after tumor evaluation, the tumor evaluation visit will be considered the end-of-study visit.
§§§§Only for patients who are candidates for tumor radical surgery. The radical resection will be arranged in the period from the 7th to the 12th week after the completion of CCRT. The international normalized ratio value will be monitored for the surgical procedure. Pathological response evaluation will be performed after tumor resection.
¶¶¶¶Patients who receive radical resection will be followed 2 weeks after surgery. This visit will be performed 2 weeks ± 3 days after surgery and patients will be evaluated with physical exam for vital signs, Eastern Cooperative Oncology Group performance status and adverse events.
####After-study follow-up will take place every 3 months ± 2 weeks and end 1 year after the end-of-study visit.
5-FU: Fluorouracil; CCRT: Concurrent chemoradiotherapy; FU1: First follow-up; FU2: Second follow-up; FU3: Third follow-up.

Patients will be followed up according to the study procedures as specified in the study protocol up to the scheduled date of study completion, or up to recovery or stabilization of a followed-up AE, whichever is later.

If possible, after the permanent discontinuation of treatment, patients will be assessed using the procedure planned for the end-of-study evaluation.

All instances of treatment discontinuation will be recorded by the investigators in the appropriate medical documents and eCRFs when confirmed.

Withdrawal from follow-up

Patients must be withdrawn from the study follow-up:

1. In case of progressive disease;
2. At the investigator's discretion;
3. At the specific request of the sponsor.

The patients may withdraw from follow-up before study completion if they decide to do so at any time irrespective of the reason, without consequences or prejudice to the patient's healthcare. The investigators may also decide to exclude them.

All withdrawals must be recorded by the investigators in the appropriate medical documents and eCRFs when confirmed.

1. If possible, the patients will be assessed using the procedure normally planned for the end-of-study visit.
2. If the patient fails to attend the end-of-study visit, the investigators should make every effort to contact the patient to identify the reason and to determine the patient's health status, including at least his or her vital status.
3. Patients who did not complete the study and for whom no end point data are available will be considered lost to follow-up.
4. Be excluded from follow-up.

Patients will be followed up for MRI tumor response evaluation and survival during the follow-up period until 1 year after the end of the study. Patients can be excluded under the following circumstances:

1. Due to progressive disease;
2. At the investigator's discretion;
3. At the specific request of the sponsor.

The patients may also withdraw from the poststudy follow-up schedule at any time, irrespective of the reason, without consequences or prejudice to their healthcare.

Discussion

The local control of cancer disease constitutes a crucial step in anticancer treatment. Radiotherapy is essential for providing rapid and effective cancer palliation. Preoperative CCRT is the standard treatment for patients with locally advanced rectal cancer because preoperative CCRT is beneficial for those patients [3–5].

Nanomedicine involves the application of nanomaterials in the diagnosis, control and treatment of disease [16]. It exploits the improved and often novel physical, chemical and biological properties of materials at the nanometer scale. It can allow early detection and prevention and improve diagnosis, treatment and follow-up. Nanomedicine is thus an interdisciplinary science involving contributions from biologists, chemists, physicists, engineers and clinicians. An engineered nanoparticle may be defined as any intentionally produced particle that has a characteristic dimension from 1 to 100 nm and has properties that are not shared by non-nanoscale particles with the same chemical composition. Two primary factors cause nanomaterials to behave significantly differently from bulk materials: surface effects (causing smooth property scaling due to the fraction of atoms at the surface) and quantum effects (showing discontinuous behavior due to quantum confinement effects in materials). These factors affect the chemical reactivity of materials, as well as their mechanical, optical, electric and magnetic properties [17].

Zhang *et al.* used various human epithelial cancer and mesenchymal cancer cell lines to evaluate the effects of PEP503 (NBTXR3) nanoparticles in the survival of cancer cell lines, including the head and neck squamous

10.2217/nnm-2022-0186

carcinoma cell lines FaDu and CAL-33, the non-small-cell lung cancer cell line NCI-H460-luc2, the colorectal cancer cell lines HT29 and HCT 116, the pancreatic cancer cell lines MIA PaCa-2 and PANC-1, the breast cancer cell line MDA-MB-231-luc-D3H2LN, the fibrosarcoma cell line HT1080 and the liposarcoma cell line LPS80T3 [10]. They demonstrated that PEP503 (NBTXR3) nanoparticles have radioenhancement properties only after activation by ionizing radiation in radiosensitive and radioresistant human epithelial cancer cell lines.

PEP503 (NBTXR3) nanoparticles have good dispersion within the tumor mass following intratumoral injection and reside at the tumor site for ≥2 weeks [10]. This property is highly relevant because radiotherapy is delivered daily at fractionated doses over 4–7 weeks for most cancers. Moreover, the product administration route is defined by the indication of interest; local injection increases the concentration in target tissue. Radiation directly penetrates tissue and cell boundaries and deposits energy heterogeneously along its path, with a higher rate toward the end. Intratumoral injection is the best option for achieving central and peripheral product distribution to concentrate nanoparticles in regions of interest. However, decreased systemic exposure probably provides the treatment with a better safety profile. PEP503 (NBTXR3) nanoparticles are radiopaque with satisfactory visualization within the tumor; diffusion in the three dimensions, including in the mass periphery and intratumoral permanency without significant surrounding leakage; thus, they are an excellent candidate for clinical application.

Hoffmann *et al.* conducted a phase I dose-escalation study to evaluate the safety profile of IMRT-activated BTXR3 nanoparticles in older patients with locally advanced squamous cell carcinoma of the oral cavity or oropharynx [12]. No DLTs or serious AEs related to NBTXR3 were noted. NBTXR3 nanoparticles were noted in the injected tumor throughout radiotherapy, and no leakage was evident in the surrounding normal tissue. Therefore, Hoffmann *et al.* concluded that the intratumoral injection of NBTXR3 nanoparticles followed by IMRT is feasible for this patient population. In a phase II–III study, Bonvalot *et al.* compared the efficacy and safety of NBTXR3 nanoparticles plus radiotherapy with radiotherapy alone in patients over 18 years of age with locally advanced soft tissue sarcoma of the extremity or trunk wall [13]. The NBTXR3 group had a significantly better pathological complete response rate than the radiotherapy-alone group (14 vs 8%; $p = 0.044$). NBTXR3 nanoparticles were thus considered a new therapeutic strategy for patients with locally advanced soft tissue sarcoma of the extremity or trunk wall. The present study will enroll patients between 20 and 80 years old because these patients can provide written informed consent by themselves. Moreover, these patients may have adequate bone marrow, renal and hepatic function and are considered to be suitable for radically curative resection. Some ongoing clinical trials are evaluating the therapeutic efficacy of NBTXR3 nanoparticles in head and neck squamous cell carcinoma (NCT01946867, NCT02901483), prostate cancer (NCT02805894) and liver cancer (NCT02721056).

Conclusion

This phase Ib/II study will be conducted to investigate the safety profile, DLT and antitumor activity of PEP503 (NBTXR3) nanoparticles with radiotherapy and concurrent chemotherapy in patients with locally advanced or unresectable rectal cancer. The potential efficacy of these nanoparticles in increasing tumor shrinkage may result in tumor downstaging, leading to increased success rates of radical tumor resection (preoperative-intention).

Author contributions

C Huang, H Hu, W Hsu, C Chen, M Huang, C Chen, P Wei, B Shen and J Wang contributed to the conception and design of the study. H Hu and W Hsu performed intratumoral injection of PEP503 (NBTXR3) nanoparticles. C Huang, C Chen, P Wei and J Wang organized the database. C Huang performed the statistical analysis. C Huang wrote the first draft of the manuscript. H Hu, W Hsu, C Chen, M Huang, C Chen, P Wei and J Wang critically revised the paper. All authors contributed to manuscript revision and read and approved the submitted version.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical disclosure

The study protocol and its amendments were approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-2013-11-07[II]), Taichung Veterans General Hospital (SF18357A) and Taipei Medical University Hospital (N202006048). Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article. This study is registered on ClinicalTrials.gov, identifier: NCT02465593.

Data sharing statement

The authors certify that this manuscript reports original clinical trial data. The data will not be made publicly available.

Summary points

Background & rationale

- Preoperative concurrent chemoradiotherapy is the standard treatment for patients with locally advanced rectal cancer.
- PEP503 (NBTXR3) nanoparticles are a suspension of inert, crystalline nanoparticles of hafnium oxide and a potential radioenhancer.
- The radioenhancement of PEP503 (NBTXR3) nanoparticles has been demonstrated in *in vivo* and *in vitro* models.
- The dose per fraction during radiotherapy could be reduced and the same therapeutic efficacy could be retained when PEP503 (NBTXR3) nanoparticles was used during radiotherapy.

Formulation of PEP503 (NBTXR3) nanoparticles

- PEP503 (NBTXR3) is a sterile, white suspension at 64 g/l, pH range 6–8, packaged in a 10 ml amber glass vial, with a rubber (bromobuthyl) stopper and sealed with aluminum-center tear-off blue seal. Each vial contains 5 ml of PEP503 suspension.

Phase Ib/II study

- The present study is a prospective, open-label, single-arm, nonrandomized study for evaluating the effects of PEP503 (NBTXR3) nanoparticles in locally advanced or unresectable rectal cancer in three institutes.
- The target population is patients with confirmed rectum adenocarcinoma (T3-4, N any or locally unresectable tumor with a distal tumor margin $\leq$10 cm from the anal verge) without evidence of distant metastatic disease, with an Eastern Cooperative Oncology Group performance score 0–1 and adequate bone marrow, renal and hepatic function.
- Patients will receive a single intratumoral injection of PEP503 (NBTXR3) nanoparticles, followed by radiation therapy. Meanwhile, patients will have concurrent intravenous infusion of fluorouracil or oral capecitabine.
- A 3 + 3 dose-escalation study design will be adopted in the escalation phase (part Ib) to identify the recommended volume of PEP503 (NBTXR3) nanoparticles for intratumoral injection.
- In the expansion phase (part II), following confirmation of the recommended volume of intratumoral injection, 18 additional patients will be enrolled at the recommended volume level for evaluations of antitumor efficacy.
- The primary end point of phase Ib is to assess the safety profile, to determine the dose limiting toxicity and to define the recommended volume (dose) of PEP503 (NBTXR3) nanoparticles.
- The primary end point of phase II is to evaluate the antitumor activity in terms of response rate.

Conclusion

- This study is the first prospective, open-label, single-arm, nonrandomized study to investigate the efficacy and safety profile of PEP503 (NBTXR3) nanoparticles with radiotherapy in patients with locally advanced or unresectable rectal cancer.

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